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Guide to Sugarcane Breeding in the Temperate Zone

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ABSTRACT

The program and practices of the U.S. Sugarcane Field Laboratory, Houma, La., are described. Discussed are sources of germplasm for breeding; evaluation of germplasm for disease resistance, sugarcane borer resistance, cold tolerance, and agronomic value; maintenance of parental material; breeding facilities and techniques; and production and storage of true seed. Index terms: naming systems, plant breeding, plant-breeding facilities, *Saccharum* spp., sugarcane, sugarcane breeding, U.S. Sugarcane Field Laboratory.

INTRODUCTION

The productivity of the sugarcane industry on the U.S. mainland is constantly threatened by diseases, insect pests, and climate. In addition, a changing economy demands types of cane suitable for mechanized culture. To insure consistent, economical production, new and improved sugarcanes are constantly needed.

The improvement of sugarcane by hybridizing different species began within or along the fringes of the Torrid Zone. The earliest and most important of these breeding sites, established between 1886 and 1913, were chosen because of their proximity to leading sugarcane-production areas and because climatic and other environmental conditions were favorable for flowering and crossing of most sugarcane forms. The spectacular success in breeding superior sugarcane varieties in the Tropics triggered the establishment of new breeding stations in other sugarcane-production areas, some within the Temperate Zones. A sugarcane station was established at Canal Point, Fla., by the U.S. Department of Agriculture in 1918, and it has been producing varieties adaptable to Temperate Zone conditions for over half a century. In 1929 the Southern Sugar Co., of Clewiston, Fla., initiated breeding work for its own plantation requirements. In 1930 the Everglades Experiment Station, Belle Glade, Fla., started cooperative breeding work with the United States Sugar Corp. (newly formed from the relict Southern Sugar Co.). After 1935 these organizations carried on independent breeding operations. Crossing work at the Everglades Experiment Station was discontinued around 1958, but seedling work was continued with true seed supplied by the Canal Point station. Breeding work is still a major research undertaking of the United States Sugar Corp.

In South Africa, a discovery of great significance to sugarcane breeding in temperate climates was made in 1947. Low temperatures during the flowering period were found to be responsible not only for the small amount of flowering but also for the low pollen fertility of most sugarcane varieties. Following these discoveries, a technique was developed for increasing flowering and pollen fertility by subjecting cut canes that were going to flower to warm greenhouse conditions. Almost simultaneously with this development, a sugarcane-breeding

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station was established on Grand Isle, La., in 1948 by the Louisiana Agricultural Experiment Station. The Grand Isle site was chosen because temperatures in late fall and early winter were warmer than those in the sugarcane-producing area. Freezing temperatures did not usually occur until midwinter. In 1950-51 two small greenhouses were built, and the flowering, crossing, and seed-production techniques developed in South Africa and India were tried with satisfactory results. However, with further improvements in control of flowering under greenhouse conditions and by artificial control of day length, the breeding work at Grand Isle was discontinued. The work thereafter was carried out in photoperiod houses and a tall crossing house located on the campus of the Louisiana State University in Baton Rouge.

In recognition of the advantages of controlled indoor breeding, a suitable greenhouse was built at the Canal Point station in 1957. In 1972 the U.S. Department of Agriculture expanded its sugarcane-breeding program, and crossing work was initiated in new facilities built by the American Sugar Cane League at the U.S. Sugarcane Field Laboratory at Houma, La., latitude 29°35′ N.

This publication deals with the important aspects of sugarcane breeding at Houma, where emphasis is being placed on obtaining new and desirable characteristics by crossing, selecting, and backcrossing from a broad genetic base, and on incorporating the best characteristics of domestic and exotic varieties from all parts of the world into single clones. The world collections of sugarcane and related genera are drawn on to provide parental material with pest resistance and other desired characteristics. Wild species of *Saccharum* in particular are drawn from worldwide sources and screened for resistance to diseases, insects, and cold, and for unique agronomic traits that may be of value in breeding sugarcane with adaptability to the Temperate Zone. Breeding procedure at Houma involves specialized techniques, including growing and care of genetic stocks in greenhouses, field plots, and can cultures; airlayering stalks of parental clones; microclimatic control in a tall greenhouse used to promote flowering and pollen production; the use of photoperiod facilities to control day length, which influences flowering and pollen fertility; and crossing to produce new hybrids or strains.

GERMPLASM SOURCES

The sugarcane genus, *Saccharum,* belongs to the family Gramineae, in the tribe Andropogoneae. Six species are generally recognized: *S. officinarum* L., *S. spontaneum* L., *S. sinense* Roxb. amend. Jeswiet, *S. barberi* Jeswiet, *S. robustum* Brandes and Jeswiet ex Grassl, and *S. edule* Hassk. However, the classification by Grassl (1974, 1977) recognizes only four species: *S. officinarum* L., *S. spontaneum* L., *S. robustum* Brandes and Jeswiet ex Grassl, and *S. sanguineum* (Grassl) Grassl. In this classification *S. sinense, S. barberi,* and *S. edule* are considered to be generic hybrids that belong in horticultural groups.

S. officinarum (2*n*=80 chromosomes) is of tropical origin. It is believed to have originated on the island of New Guinea, where it presumably evolved from *S. robustum* under long-continued selection by native gardeners. *S. officinarum* is characterized by thick juicy stalks, flamboyant stalk colors, relatively high sugar content, wide leaves, and low fiber content. Varieties of this species, variously referred to as "thick-stemmed tropical," "original," or "noble" sugarcanes, are essentially cultivated plants that cannot survive in the wild. This species provides the indispensable sugar-bearing component to essentially all interspecific hybrids, as well as high weight per cane and self-trashing of cane. Resistance to such formidable sugarcane diseases as smut, Fiji disease, and downy mildew is found in representatives of the species. Hybridization of *S. officinarum* and other species except *S. robustum* is difficult.

S. spontaneum (2*n*=27-128 chromosomes) clones form a highly complex group, and they have a rather wide indigenous range. Forms are known from Borneo, Burma, Celebes (Sulawesi), China, Java, New Guinea, the Philippines, Taiwan, and the Indian Subcontinent and north and east Africa. These wild sugarcanes range from dwarf bushy types about 1 meter in height, with wiry leaves and slender stems, to erect broad-leaved types 6 meters tall, with relatively thick stems. The stems, or stalks, are hard, very pithy, and often hollow in the center, with a low sugar content and little juice. Most have clinging leaf sheaths and an erect growth habit, but others send out trailing adventitious stems, and some produce under-

ground stems, or rhizomes. They grow and thrive vigorously under natural conditions over a large geographical area, from about latitude 8° S. to 40° N. *S. spontaneum* has become more important than any other species in the genus except *S. officinarum* in recent years because its hybridization with *S. officinarum* is the first step in the nobilization[2] programs by which modern commercial varieties are produced.

S. sinense and *S. barberi* (2*n*=82-124 chromosomes), cultivated forms occurring in subtropical India and adjacent parts of China, were recognized as close relatives to each other and to the wild *S. spontaneum* by Jeswiet (1925). On the other hand, it has been theorized by Parthasarathy (1946) that *S. sinense* and *S. barberi* originated as natural hybrids between indigenous Indian forms of *S. spontaneum* and *S. officinarum* transported by man in remote times. However, Grassl (1977) states that some of the native north Indian sugarcanes originated before *S. officinarum* arrived there. *S. sinense* is tall, with relatively broad leaves, whereas *S. barberi* is short, with narrow leaves. The stalks of both species are relatively thin and fibrous, and some forms, such as 'Chunnee' and 'Uba', have been successfully used in breeding commercial varieties.

S. robustum, like *S. spontaneum,* occurs in the wild, but its range is much more limited, apparently being confined to New Guinea and nearby islands. *S. robustum* differs from *S. spontaneum* by its typically greater stalk height and stalk diameter. Like those of *S. spontaneum,* its stalks are tough, fibrous, and generally low in sugar, but a few of the natural clones reportedly have juice of relatively high Brix. The stalks are erect or reclining and unbranched, always with the greatest diameter near the nodes with a swollen growth ring. In its natural habitat, *S. robustum* is often extremely vigorous, forming compact tufts or dense canebrakes up to 10 meters tall. This species has been used in breeding work in several countries, with varying success. *S. robustum* as typified by the clone NG 28-251 and similar types from New Guinea, New Britain, and the New Hebrides has 2*n*=80 chromosomes, but there are also forms on the north coast of New Guinea, hybrid types involving *Miscanthus* species, that have 2*n*=60.

S. sanguineum, in Grassl's classification (1974, 1977), is typified by NG 28-219, which has blood-red tissues in the culms, particularly near the rind; deep-red buds, dewlaps, and growth rings; and 2*n*=60 chromosomes.

S. edule, being sexually sterile, is of no importance in sugarcane breeding. Restricted to New Guinea and neighboring islands, it is characterized by pubescent leaves and the production of peculiar swollen and aborted inflorescenses.

Present-day commercial varieties were developed from only a few original forms of the genus *Saccharum.* Yet, two world collections of sugarcane, one maintained by the U.S. Department of Agriculture at Canal Point, Fla., and the other by the Indian Sugarcane Breeding Institute at Coimbatore, are available to sugarcane breeders. Since relatively few representatives of the genus have been used extensively in breeding, much remains to be done in systematically surveying the material available to identify superior new sources of breeding material.

The world collections also have various grasses from genera closely related to sugarcane. One or more species of *Ripidium, Miscanthidium, Miscanthus, Sclerostachya, Eccoilopus,* and *Sorghum* have been successfully hybridized with *Saccharum,* and there is widespread interest in the possibility of utilizing intergeneric hybridization in breeding new and improved sugarcanes.

Many outstanding commercial and near-commercial sugarcane varieties from different breeding stations around the world are also included in the world collections. These varieties are the result of long-continued selection for important agronomic traits and resistance to diseases, insect pests, and unfavorable environmental conditions. Each variety is a single clone of interspecific derivation that has been propagated vegetatively. The ease with which sugarcane may be propagated asexually permits the breeder to rapidly exploit an outstanding genotype, and though excellence as a sugar

[2]The term "nobilization" is derived from "noble" as that word has historically applied to *S. officinarum,* the species whose stalks have a "noble" appearance—large barrel size, tall, brightly colored. The first interspecific crosses between *S. officinarum* and *S. spontaneum* produced progenies that resembled the *S. spontaneum* parents. Subsequent backcrosses were made with the F_1 progenies as nonrecurrent parents and other clones of *S. officinarum* as the recurrent parents. This step was continued for 3 or 4 cycles of backcrossing. The clones selected at each stage of backcrossing were said to be nobilized because with each cross the progenies more resembled their "noble" ancestry.

producer is not always a reliable indication of the potential value of a sugarcane clone as a parent, the best parents are generally those that most strongly exhibit the desired traits.

Naming systems for sugarcane clones are important in maintaining identity, and symbols frequently encountered by breeders are listed in table 1. When new projects are started, the list can be consulted to insure that any new numbering system does not use symbols already in use.

PRELIMINARY EVALUATION OF GERMPLASM

At the U.S. Sugarcane Field Laboratory germplasm is evaluated for resistance to diseases, the sugarcane borer, and cold, and for desirable agronomic characteristics before it is included in the breeding program. Testing, which is carried out in the greenhouse, field, and laboratory, may take from a few months to several years before a clone is declared superior for one or more desired characteristics.

Disease Resistance

The most serious sugarcane diseases in Louisiana today are mosaic and ratoon stunting disease (RSD). Mosaic is caused by a virus (potyvirus group), and for many years it was thought that a virus was the causal agent of RSD, but diagnostic tests (Gillaspie et al. 1973) indicate that the disease may be caused by a bacterium as yet unnamed. Both mosaic and RSD cause significant losses in sugarcane yields, especially in combination. Red rot, caused by *Physalospora tucumanesis* Speg., and pythium root rot, caused by *Pythium arrhenomanes* Drechsl., are also important, but their status has remained unchanged during the last decade. Sugarcane rust, caused by *Puccinia melanocephala* H. and P. Sydow, was found in 1979 in Florida, Louisiana, and Texas; sugarcane smut, caused by *Ustilago scitaminea* Sydow, was found in 1978 in Florida and in 1981 in Louisiana.

S. spontaneum has long been recognized as the basic source of resistance to mosaic in the breeding of commercial sugarcanes. Yet recent investigations show conclusively that resistance to the disease in this wild species is far from universal. On the other hand, resistance to mosaic in clones bred from the established lines is still in evidence. Therefore, screening for mosaic resistance in potential parental material is not confined to the wild species.

In evaluating clones for mosaic resistance in the greenhouse, single-bud cuttings of each clone to be tested, along with controls, are presprouted in an incubator at about 32° C before being planted upright in 7.6-cm peat pots. Ideally, 3 replications of 10 plants of each clone in a test are randomized on the greenhouse benches; the benches are covered with about 6 cm of sand before the pots are set in place. When most of the plants are about 15 cm tall, they are inoculated with a mixture of mosaic strains, usually strains H and I, which are most prevalent in Louisiana. The inoculum is prepared according to the recommended procedure (Todd 1961, Breaux and Tippett 1963) and administered by a spray technique (Dean 1960, Bird 1961). The plants are carefully observed for about 3 months (sometimes longer with *S. spontaneum* clones, which are slow to express symptoms). Only the most resistant clones are kept, and these, especially the *S. spontaneum* clones, may be tested for resistance repeatedly before giving them to the breeding program.

To increase the frequency of mosaic resistance in interspecific hybrid populations, young seedlings are evaluated while they are growing in the greenhouse. Again, the seedlings are sprayed with a mixture of virus strains H and I. Up to 90% or more of the seedlings are frequently found to be susceptible. All mosaic-infected seedlings are discarded; those free of mosaic symptoms are set in the field. Evaluation for resistance to the virus is continued in the original single-stool field nurseries, in single-row, 1.8- and 4.6-meter clonal plots (first and second line trials, respectively), and in greenhouse single-bud inoculation tests.

In single-stool nurseries, which may contain up to 100,000 individually planted seedlings, infected plants were in the past destroyed in the spring by spraying with a herbicide, but with an increased incidence of mosaic in the nurseries, this method of roguing became impractical. Now, mosaic-infected stools are carefully noted and discarded at selection time in the first-ratoon crop. Only mosaic-free canes are replanted in the line trials.

In the first and second line trials, all clones are carefully observed for mosaic symptoms in both spring and fall. Separate nurseries of

Table 1.—Naming systems for sugarcane[1]

Symbol	Example	Explanation
B	B 147	Barbados, selection 147 (WI in old numbering system).
B	B 35-9	Bourne, 1935, selection 9 (bred by B. A. Bourne, Clewiston, Fla.).
BA	BA 11569	Barbados, selection 11569 (bred by J. R. Bovell).
Bar	Bar 175	Baraguá (Cuba), selection 175.
BH	BH 10/12	Barbados Hybrid, 1910, selection 12.
BJ	BJ 5924	Barbados-Jamaica, 1959, selection 24 (Barbados fuzz, Jamaican selection; current system).
BL	BL series	Barbados (fuzz), Lyallpur (Pakistan) series.
BR	BR 62-56	Barbados-Romana, 1962, selection 56 (Barbados fuzz; central La Romana Province, Dominican Republic, selection; current system).
BT	BT 691020	Barbados-Trinidad, 1969, selection 1020 (Barbados fuzz; Caroni Ltd., Trinidad, selection).
C	C 35	Cuba, selection 35 (Agricultural Experiment Station, Santiago de las Vegas, Cuba).
C	C 278	Queensland alphabet series, 1941, Badila × *S. robustum* (NG 28-251).
CA	CA 3874	Central Aguire (Puerto Rico), 1938, selection 74.
CAC	CAC 57-60	College of Agriculture Cane, 1957 selection 60 (University of the Philippines, Los Baños).
CB	CB 40-13	Campos, Brazil, 1940, selection 13.
Cl	Cl 41-223	Clewiston (Florida), 1941, selection 223 (U.S. Sugar Corp.; current system).
Co	Co 7010	Coimbatore (India), 1970, release No. 10 (new series, begun in 1962).
CoJ	CoJ 39	Coimbatore (fuzz)-Jullundur (East Punjab), selection 39.
CoK	CoK 30	Coimbatore (fuzz)-Karnal (India), selection 30.
CoL	CoL 9	Coimbatore-Lyallpur (Pakistan), selection 9.
CoR	CoR	Coimbatore-Risalewala (India?).
CoS	CoS 648	Coimbatore-Shajahanpur (India), 1964, release No. 8.
CP	CP 65-357	Canal Point (Florida), 1965, selection 357 (U.S. Department of Agriculture; current system).
CR	CR 63-113	Central Romana, 1963, selection 113 (Dominican Republic, current system).
D	D 74	Demerara, selection 74 (Guyana, current system).
E	E 16	Egypt, selection 16.
EK	EK 28	E. Karthaus, selection 28 (Java).
EPC	EPC 279	Estación Palmira, Colombia, selection 279.
F	F 146	Formosa, selection 146 (Taiwan current release series).
F	F 36-819	Florida, 1936, selection 819 (Everglades Experiment Station, University of Florida).
Fiji	Fiji 11	Fiji (originals), clone 11 (collection of wild and noble clones made by Colonial Sugar Refinery commencing in 1951).
H	H 59-3775	Hawaii, 1959, selection 3775 (Hawaiian Sugar Planters Association; current system).
HQ	HQ 64-95	Hambledon, Queensland, 1964, selection 95 (Colonial Sugar Refinery, new series begun in 1961; current system).
I	I 227/55	Ishardi (Bangladesh), selection 227, 1955.
IA	IA 3274	Indo-American, selection 3274 (clones bred and selected in India for the U.S. Department of Agriculture).
IAC	IAC 50/134	Instituto Agronomico de Campinos, 1950, selection 134 (Brazil; current system).
IANE	IANE 55-33	Instituto Agronomico de Nordeste, 1955, selection 33 (Brazil).
ICA	ICA 69-102	Instituto Colombiano Agropecuario, 1969, selection 102 (Colombia; current system).
ID	ID 36	Ishardi (Bangladesh), selection 36 (Sugar Cane Experiment Station; current system).
JAR	JAR 58-6-23-6	H. G. Sorensen, Central Jaronu (Cuba), 1958, selection 6-23-6.
L	L 116	Lyallpur (Pakistan), selection 116.
L	L 60-25	Louisiana, 1960, selection 25 (Louisiana State University; current system).
M	.....	*See* MPR.
M	M 1049/70	Mauritius, selection 1049, 1970 (current system).
MB	MB 1/64	Mauritius Breeding (series), selection 1, 1964 (Mauritius Sugar Industry Breeding Series, Research Institute; current system).
Mer	Mer 61-12	Meridian (Mississippi), 1961, selection 12 (U.S. Department of Agriculture; current system; "Mer" also used for sorghum).
Mex	Mex 57-1285	Mexico, 1957, selection 1285 (Instituto para el Mejoramiento de la Producción de Azucar (IMPA); current system).
ML	ML 318	Media Luna (Cuba), selection 318 (bred by Ricardo Beattie).

See footnotes at end of table.

Table 1.—Naming systems for sugarcane[1]—Continued

Symbol	Example	Explanation
MPR	MPR 28	Mayagüez, Puerto Rico, selection 28 (same as M series (synonym: M28); U.S. Department of Agriculture).
MQ	MQ 60-1469	MacKnade, Queensland, 1960, selection 1469 (Colonial Sugar Refinery, Australia).
MY	MY 5329	Mayarí, selection 5329 (Estación Experimentale de Mayarí, Oriente Province, Cuba).
N	N 6	Natal (South Africa), selection 6 (seed also produced in South Africa).
NA	NA	Nova, Argentina (replaces the VA series).
NCo	NCo 310	Natal-Coimbatore, selection 310 (Combiatore, India, fuzz; Natal, South Africa, selection; South African Sugar Association).
NG	NG 28-251	New Guinea, 1928, original clone 251 (U.S. Department of Agriculture expedition).
NG	NG 51-140	New Guinea, 1951, original clone 140 (Bureau of Sugar Experiment Stations, Australian expedition).
NG	NG 57-259	New Guinea, 1957, original clone 259 (U.S. Department of Agriculture/Hawaiian Sugar Planters Association expedition).
NH	NH 70-1	New Hebrides, 1970, collection 1 (a collection made by the New Hebrides Department of Agriculture in 1970 for the International Society of Sugar Cane Technologists Germplasm Committee).
PB	PB	Pernambuco, Brazil.
PB	PB 929	Plant Breeding, selection 929 (College of Agriculture, the Philippines; used before CAC, which see).
PB	PB 51-20	Palm Beach (Research Farm), 1951, selection 20 (Florida, U.S.A.).
PM	PM 20	Potrero, Mexico, selection 20 (=PO Mex 20).
POJ	POJ 2878	Proefstation, Oost Java, selection 2878.
Pop	Pop 56-14	Poplarville (Mississippi), 1956, selection 14 (U.S. Department of Agriculture; current system).
PPQK	PPQK	Synonym of Pepe Cuca and Bar 114/35; variety discovered by José Sanchez, Camagüey, Cuba.
PR	PR 61-53	Puerto Rico, 1961, selection 53 (current system).
PSA	PSA 14	Philippine Sugar Association, selection 14.
PT	PT 51-204	Ping Tung, 1951, selection 204 (Sugar Research Institute, Taiwan; current system).
Q	Q 90	Queensland, selection 90 (Queensland, Australia, Bureau of Sugar Experiment Stations, current system).
R	R 550	Réunion, selection 550 (release series, current).
RK	RK 63-1	Ryukyu, 1963, selection 1 (Okinawa, Japan; current system).
SC	SC 12(4)	Saint Croix (U.S. Virgin Islands), 1912, selection 4.
SES	SES 6	*Spontaneum* Expedition Scheme, selection 6 (original *S. spontaneum* clones collected by expeditions sent out by Indian Government; includes clones belonging to related genera).
SW	SW 111	Sempel Wadak, selection 111 (a sugar factory in Java).
TA	TA 7	Taiwan (Sugar Manufacturing Co.), selection 7.
TUC	TUC 68-19	Tucumán (Argentina), 1968, selection 19 (current system).
UCW	UCW 53-29	United Fruit, Cuban-American, and West Indies Group, 1953, selection 29.
US	US 52-13-1	United States, 1952, cross or import 13, selection 1 (U.S. Department of Agriculture; current system).
VA	.....	*See* NA.
VMC	VMC 67-510	Victorias Milling Co. (Negros, the Philippines), 1967, selection 510 (current system).
WI	.....	*See* B.
.....	Pindar[2]	Colonial Sugar Refinery, Australia.

[1]For more complete information refer to Grassl (1953) and Daniels (1971).
[2]Names of historical and mythological characters are used.

clones selected from commercial and basic breeding lines are maintained. Mosaic occurs in both classes of genetic material, having been spread by insects from surrounding fields of infected plantation cane. In commercial progeny (highly inbred from selected old-line interspecific hybrids of similar genetic makeup) clones with mosaic infection in the range of 25% to 100% are flagged with brightly colored surveyor's tape, and these are discarded in the final selection. Those with less than 25% mosaic will be selected if they appear to be outstanding agronomically. In the basic progeny (bred from widely diversified new-line germplasm resources) only clones completely free of mosaic symptoms are selected.

Clones with commercial potential in the first-ratoon crop of the second line trials are assigned CP (Canal Point, Fla.) numbers[3] and planted in a two-replicate nursery in a randomized complete-block design of single-row plots 4.6 meters long. Early-generation hybrids from basic breeding lines are assigned U.S. breeding numbers and planted in 3-meter single-row plots in a special nursery. Plots in both nurseries are inspected three or four times during the growing season for mosaic. Clones in these plots are subjected to additional inoculation tests in the greenhouse and to natural infection in field tests located in areas where the incidence of mosaic is high. Furthermore, evaluation of the commercial germplasm for mosaic resistance is routinely carried out in variety-yield trials on farms around the Louisiana sugar belt.

Testing for varietal reaction to RSD is primarily a matter of determining relative tolerance because fully resistant varieties, at least among commercial selections, have not been encountered. RSD tends to reduce the yield of infected cane, especially in the ratoon crops; therefore, the method of determining tolerance is to compare the weight of cane grown from seedcane that has been freed of the bacterium by heat treatment (hot air at 58° C for 8 hours, or hot water at 50° C for 2½ to 3 hours) with that of similarly treated cane that has been reinoculated with the bacterium. (The first progeny of heat-treated cane rather than the heat-treated cane itself is used in order to eliminate the effect of the heat treatment on germination and growth.) Inoculation consists of dipping freshly cut ends of the seed piece in juice extracted from RSD-infected cane. Four or five replications of three-row plots 6.1 meters long are planted with the seedcane from each treatment. Yields are determined from plant-cane and first- and second-ratoon crops. Reaction among clones ranges from extreme susceptibility and intolerance as indicated by a loss of yields in excess of 25% to a high degree of tolerance with little or no loss of yield. Canes highly tolerant of the disease are preferred for breeding.

Because of the development of resistant varieties, red rot and pythium root rot are not now of major concern in Louisiana. While artificial inoculation techniques have been developed to test varietal reaction to these diseases (see Abbott et al. 1965), a stringent test for varietal reaction takes place in the test fields. Here, the susceptible varieties are simply eliminated by natural infection of the seedcane and hence are overlooked in the selection process.

Screening of sugarcane clones for resistance to smut, one of the most devastating diseases of sugarcane, is an important undertaking in many countries, including the United States. Screening is now underway in Florida to identify resistance in both commercial varieties and clones to be used in breeding. Seed pieces of each clone to be screened are immersed for 10 minutes in a suspension of 5 million spores per milliliter of water and then planted in a field near a heavily infected commercial plantation. Ratings for resistance are made in conformity with the procedure used in Hawaii (Ladd and Heinz 1976).

Sugarcane Borer Resistance

The sugarcane borer, *Diatraea saccharalis* (F.), is the most destructive insect pest of sugarcane in Louisiana. This has led to increased testing in recent years to evaluate and identify superior commercial and unexplored germplasm to breed improved borer-resistant sugarcane varieties.

In initial tests to determine borer resistance, clones from both commercial and basic crosses were planted in the field for comparison with borer-resistant commercial varieties, the latter being used as controls (Jackson et al. 1971). Throughout the growing season all varieties in

[3]See table 1.

the tests were repeatedly infested with borer egg masses produced in the laboratory. At harvest, when the stalks were examined for bored joints and extent of damage, the selections from basic crosses, while not as advanced agronomically as selections from commercial crosses, showed greater resistance to the borer under the conditions of the test. The resistant material has since been used extensively in crosses at Houma.

Greenhouse screening of juvenile seedlings for borer resistance is not recommended. At Houma (unpublished data) and at Louisiana State University (Pan and Hensley 1973), progenies of both basic and commercial crosses were grown first in greenhouse flats, where they were artificially infested with two first-stage larvae per seedling. When planted in the field, the few surviving seedlings proved to be no more resistant to borer attack than unscreened seedlings.

In another test, 31 *S. spontaneum* clones used in breeding work were grown in containers in the greenhouse and out of doors, and were artificially infested with borer egg masses produced from laboratory-reared moths (Jackson and Dunckelman 1974). When the stalks were sufficiently mature, they were dissected, and borer damage was assayed. Two of the clones appeared to be highly resistant, and one, which flowers freely at Houma, has already been used to start a new breeding line.

The most advanced varieties that have shown borer resistance are planted annually at three sugarcane farms in south Louisiana. Entries in these trials include varieties currently being grown by Louisiana farmers plus any candidate varieties with commercial potential for Louisiana. A randomized complete-block design is employed at all locations, with each varietal entry replicated four times. Individual plots consist of four rows, each 9.75 meters long. Resistance or susceptibility is evaluated at harvest by determining for each variety the percentage of internodes bored. Twenty-five stalks are selected at random from each plot and scanned for the presence of borer entrance and exit holes. Insecticides are not applied in most trials, thus permitting natural infestation to exert selection pressure on the varietal entries. However, in some trials, insecticide applications are occasionally made late in the crop season for control of heavy infestations, especially when there is a potential for 50% or more of the internodes to be tunneled. Varieties found to be resistant are returned to the breeding program.

At present, the diversity of genetic material, the means of applying uniform selection pressure, and demonstrated selection efficacy suggest that the development of borer-resistant sugarcane may be possible.

Cold Tolerance

The development of cold-tolerant sugarcanes for the Temperate Zone has long been a goal of sugarcane breeders. Yet, early attempts to develop cold-tolerant sugarcanes by crossing 'Turkestan' (*S. spontaneum*) with 'Otaheite' (*S. officinarum*) and 'POJ 2878' (a tropical hybrid) met with failure in the late 1930's. The use of the tropical canes as recurrent parents probably accounted for the rapid loss of cold tolerance needed in the Temperate Zone. Now, with new techniques for evaluating cold tolerance in sugarcane and the discovery of additional cold-tolerant *S. spontaneum* clones, the development of clones with a greater degree of cold tolerance is possible.

At Houma, sugarcane is evaluated for cold tolerance in both field and laboratory tests. The chance of field testing being successful is limited by the erratic occurrence of freezes and the variability of freeze duration, temperature, and postfreeze conditions. Little cold tolerance has been found in seedlings from commercial sugarcane parents in the field. However, cold tolerance has been found in a few selections from *S. spontaneum* lines that survived two successive freezes of -3.3° C of approximately 4 hour's duration. Freeze-damage symptoms were allowed to become well developed, and the canes were rated for cold tolerance based on the estimated percentage of green tissue in the leaves, the condition of lateral buds, and freeze damage to stalk tissue. The latter condition is difficult to evaluate in pithy wild canes. In a test with 32 can-cultured *S. spontaneum* clones exposed to natural freezes at Houma, Dunckelman and Breaux (1969) found that some clones were cold tolerant while others were very susceptible, but none were as susceptible as the commercial varieties used in the test. The *S. spontaneum* species shows definite promise as a source of cold tolerance for breeding.

In a subsequent experiment Irvine (1978) artificially froze various clones under controlled conditions in the freezer. He found that cold tolerance greater than that present in commercial varieties is available in *Saccharum* and several related genera. These limited tests suggest that more forms with even greater cold tolerance may be found in the world collections of sugarcane and related genera.

In another approach, Breaux and Irvine (1973) artificially froze young, actively growing seedlings at -5.5° C for 8 hours in a large thermostatically controlled freezer. The surviving seedlings (about 10%–15%) were planted in the field in a paired comparison with the unfrozen seedlings from the same cross. Following a -5.0° C freeze in early winter, there was no difference in frequency of cold-tolerant types between the two treatments. The frequency of cold-tolerant clones could be increased by careful parent selection, but not by freezing young seedlings and selecting survivors.

A major goal of contemporary sugarcane breeding, especially in the Temperate Zone, should be the collection, screening, and incorporation of germplasm transmitting superior cold tolerance.

Agronomic Value

The heritable traits of greatest importance to sugar production include number of stalks per unit area of land, stalk diameter and height, resistance to lodging, brittleness, Brix (total soluble solids), and percentage of sucrose in the juice. Evaluation of clones for superior agronomic traits begins in the seedling stage and continues in first and second line trials and in replicated plots grown at the U.S. Sugarcane Field Laboratory, at Louisiana State University, and at strategic locations throughout the Louisiana cane-growing area. Selectors look not only for clones that equal or exceed control varieties in overall agronomic value, but also for clones with specific agronomic traits that may be of value in breeding, even though they may appear to have little chance of ever becoming commercial canes. Throughout the long and detailed selection procedure, every commercial-type and basic-line cane is looked upon as a potential parent variety. The truly superior selections are used in the breeding program as soon as it is felt that they have been adequately evaluated for one or more desired agronomic traits. The ability of a clone to transmit a particular trait can only be determined experimentally; thus, it is the custom to list parents that pass on particular traits rather than to list genetic information.

In both basic and commercial breeding lines the first selection for agronomic traits is done in the first-ratoon, single-stool nursery. Because of low repeatability for most of the selection criteria at this stage, liberality is exercised in evaluation in single stools. However, the single stool is still compared as a whole to commercial varieties grown from single-bud cuttings planted throughout the nursery.

Clones from the seedling progenies are further evaluated for agronomic traits in the ensuing first and second line trials and in a replicate nursery (two 4.6-meter single-row plots) of the best clones selected from second line trials. At each stage of testing, new clones are compared with commercial controls. In progenies involving new basic germplasm, where one or both parents may be a clone of *S. spontaneum, S. robustum, S. officinarum,* or a species of some genera related to *Saccharum,* the selections do not have to meet all commercial standards; rather, they are the best the progeny has to offer in the trait for which the cross was made, for example, mosaic resistance, borer resistance, cold tolerance, or stalk number. Clones selected from first-ratoon plots of second line trials from basic crosses are given to the breeding program and replanted in 3-meter single-row plots in a special nursery; the few clones that meet commercial requirements are also sent to the "infield" testing program, along with commercial types selected from conventional lines.

In the "infield" testing program (plots 5.5 by 6.1 meters, replicated three times) potential breeding value for agronomic improvement is considered along with agronomic traits of commercial importance. Throughout the growing season varieties are independently observed by four agronomists, who rate each clone for stalk population, stalk diameter, stalk height, erectness, brittleness, and estimated tons of cane per unit of area. Ratings range from 1 to 10. Five is assigned to the standard variety included in the planting. An average rating above 4 indicates sufficient vigor to warrant further evaluation.

Average sucrose content of the juice (as a percentage of the standard) is also used in selection of varieties. In advanced stages of testing, yield of cane per unit of area is determined by weighing all cane harvested from a plot. Sucrose content is determined by sampling cane from each plot. All yield data are combined and used in selection of varieties for further evaluation. The clones with superior agronomic characters are sent to the "outfield" testing program, and those which appear to have outstanding traits of importance in breeding are given to the breeding program.

"Outfield" testing is the final evaluation before varieties are released. Each variety is planted in 4 plots 3 rows wide (1.8 meters apart) by 9.7 meters long at 14 locations representing the major soil types and climatic regions of the Louisiana sugar industry. Each variety is evaluated for agronomic traits in essentially the same manner as in the infield tests, except that testing is done on a much larger scale and with greater precision. It is during this stage of testing that many of our most valuable breeding canes are identified.

MAINTENANCE OF PARENTAL MATERIAL

Field Nursery

New clones intended for use as parents are given to the crossing program late in the year, soon after they have been tested for one or more characteristics deemed of value in breeding sugarcanes with adaptability to temperate climatic conditions. As soon as possible, usually on the day received, the clones are planted directly in the field nursery in small unreplicated plots. Plantings are usually made between late summer and midfall because during this time newly evaluated clones are returned to the crossing program from various research projects allied with the program. The nursery plots are 3 meters long, with 0.6-meter alleys. The rows are 1.8 meters wide, the standard row width in Louisiana sugarcane fields. Four stalks of each clone are planted per plot and covered with soil to a depth of about 10 cm. The plots are then sprayed with herbicide to control winter grasses and broadleaf weeds.

When the canes start growing in the spring, the plots are fertilized, cultivated, and sprayed with herbicides and insecticides as needed, in accordance with plantation practices. In early fall, the mature stalks of each clone in the nursery are counted to determine the supply of seed cane and to again rate the clones for such criteria as stalk diameter, tillering, and erectness, and freedom from mosaic, borer damage, and pith. Each year, on the basis of field observations, greenhouse tests, and small-mill tests, new clones are added to the nursery and others dropped. Every 4 years, the most outstanding clones in the nursery are replanted, and the old nursery is plowed out. In this manner, the acquisition and disposal of parental material is continued indefinitely. Floral initiation and development of the clones is noted.

Greenhouse and Can Cultures

Select breeding clones to be used in crosses are planted in flats of soil in the greenhouse about 1 year in advance of each crossing season (fig. 1). Single-bud cuttings of each clone are planted in 38- by 61-cm metal flats in the seedling greenhouse in early fall. The flats are filled to the top with a suitable planting mixture. The single-bud cuttings are planted upright in the flats with the upper portion of the cuttings

Figure 1.—Single-bud cuttings of breeding clones planted in flats inside greenhouse. After about 4 months, the small stools will be ready for transplanting in garbage cans.

extending about 4 or 5 cm above the soil level. The plants are watered daily, and when well established, they are fertilized, sprayed with insecticide, and clipped back at intervals to prevent spindliness and to insure good but regulated growth. Such treatment encourages tillering and strong stocky plants with short internodes on the basal part of the shoots.

After about 4 months in the greenhouse flats, the small stools of cane are ready for transplanting to 37.8-liter garbage cans. The insides of the cans are painted beforehand with asphalt varnish to prevent zinc toxicity to young, tender roots. The plants are removed from the flats with some soil adhering to the roots and are immediately transplanted in the cans, which are placed on carts and the floor of the crossing greenhouse (fig. 2). The soil mixture in the cans is made up of 3 parts field soil, 2 parts coarse construction sand, and 2 parts sphagnum peat. The stools are set deeply into the soil so that about 13 cm of the above-ground basal portion of each stool, with many buds, is placed well below the soil surface in each can. If the plants are weak, two or three are planted in each can. This method of transplanting insures prolific tillering and the ultimate availability of many large stalks per can during the crossing season.

From the time of transplanting to cans in midwinter to midspring, the can cultures remain in the warm greenhouse. Then, when danger of frost is past, some of the cans are moved from the greenhouse to the carts in the photoperiod house and some to stationary outdoor racks, while others are left in place on the carts of the greenhouse to be moved outside when the temperature is right. Only early-flowering canes are moved to racks outdoors since they usually encounter very little cold weather before setting floral initials in the fall. The later, more difficult flowering canes, which require special treatment to bring about flowering in the temperate Louisiana climate, are kept in the crossing greenhouse and photoperiod house.

All can cultures are watered during the growing season by means of a system of plastic pipes permanently affixed to metal tie racks just above the tops of the metal cans (fig. 3). Above each can a 1.6-mm hole in the pipe provides a water outlet. The pipes are connected to each other and to water faucets by flexible rubber hoses, which are easily coupled and uncoupled by means of twist-lock connecters. This method, more efficient than watering by hand, enables attendants to continue with other work while the cans are being watered. The only disadvantage of the system is that all cans are watered

FIGURE 2.—Four-month-old cane in garbage cans on rail carts in the tall greenhouse. Transplanted in the cans in midwinter, the stools will remain in the greenhouse until danger of frost is past.

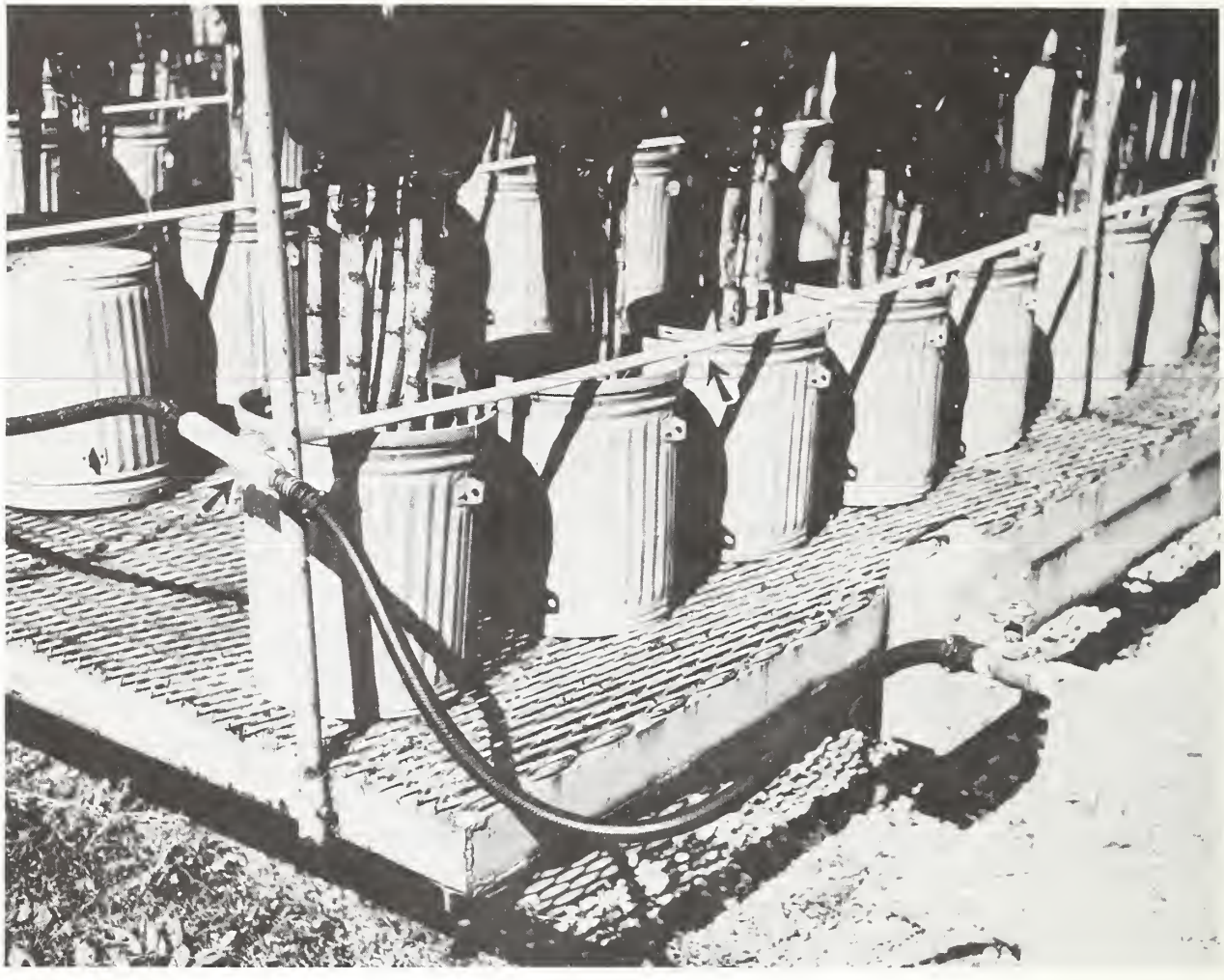

FIGURE 3.—Hose-and-pipe system for watering can cultures.

uniformly, whether they need water or not, but this problem has been largely overcome by using a porous planting mixture and by placing about 8 cm of coarse gravel in the bottom of each can. When water is found standing in cans, a steel probe is used to punch drain holes through the soil and bottoms of the cans. New roots soon fill the drain holes, and the flow of water from the cans is regulated.

When the can-cultured stools of cane are about 1.5 meters tall, weak shoots and dead leaves are eliminated by hand, and the process of elimination is continued until only the largest, most uniform stalks remain. As the canes grow taller, they are tied with cotton or nylon ropes (1.8 m long and 1.3 cm in diameter) to the steel tie bars of the racks to maintain erectness and prevent the cans from being toppled by wind.

Throughout the growing season, the can cultures are fertilized lightly every 2 weeks with a suitable complete fertilizer with micronutrients. Repeated fertilizations are required because of the leaching effect of daily watering. When more rapid growth of the plants is desired, the application of fertilizer is doubled. In addition to the frequent fertilizations, the canes are also sprayed every 2 weeks during the growing season with an insecticide recommended for control of sugarcane insects.

BREEDING WORK AND CYTOLOGY

The primary goal of the basic breeding program at the U.S. Sugarcane Field Laboratory is

to broaden the narrow genetic base upon which contemporary varieties have been bred. New germplasm from various *Saccharum* species and related genera is gradually being assimilated into commercial breeding lines through a long-range process of planned introgression.

In crossing, special emphasis is placed on the use of wild *S. spontaneum* as a donor for hardiness and resistance to diseases and insects; on *S. robustum* for large size, erectness, and vigor; and on *S. officinarum* and commercial breeding canes for juiciness, high sucrose content, low fiber content, and general adaptability for meeting commercial requirements in field and factory. Clones or hybrid derivatives of various genera related to sugarcane are also included in crosses for characteristics not found in *Saccharum*. Attempts are underway to incorporate the desirable features of the parents into a composite in a relatively short time.

Selected clones of the wild *Saccharum* species and their wild relatives in other genera are being nobilized in a systematic regimen of crossing, selection, and backcrossing in which the *S. officinarum* clones and selected interspecific commercial breeding canes are used as recurrent parents. The most highly nobilized selections—as they approach or exceed commercial standards for agronomic worth and resistance to such factors as mosaic, cold, and the sugarcane borer—are immediately sent to cooperating breeding programs for further exploitation in crosses designed to produce superior sugarcanes with Temperate Zone adaptability.

The ultimate goal is to breed new commercial sugarcanes that have some or all of the following desired traits: resistance to diseases and insect pests; drought, cold, and salt tolerance; adaptability to mechanized culture, harvest, and handling; longer and better ratooning capacity; higher yields of cane per unit of area; more sugar per ton of cane; and consequently more sugar per unit of area. Achievement of these breeding goals depends basically upon the production of an adequate and continuing supply of true seeds from genetically diverse crosses made with superior parent varieties. While strict Mendelian principles of genetics are not entirely applicable in interspecific hybridization to attain our goals in sugarcane breeding, a basic understanding of these principles is helpful in detailing any sort of scientific approach to the strategy of breeding improved sugarcane varieties.

Sugarcane varieties as we know them today are complex interspecific hybrids of several *Saccharum* species. The main contribution of genetic material to most commercial varieties came from *S. officinarum* and *S. spontaneum*, with lesser contributions from *S. sinense* (mainly through 'Chunnee') and *S. robustum*. Early varieties, such as 'POJ 2878' that resulted from interspecific hybridization between *S. officinarum* and Kassoer (itself a natural interspecific hybrid of *S. officinarum* × *S. spontaneum*), revolutionized the failing, disease-ridden sugar industries of the Tropics. These industries had previously depended strictly on clones of *S. officinarum* (noble) canes for production. Hardiness and disease resistance were gained in 'POJ 2878', and similar varieties which followed, by combining *S. spontaneum* and *S. officinarum* chromosomes.

Sugarcane species are all believed to be natural allopolyploids which arose as hybrids between earlier closely related forms that may no longer exist. The role of interspecific hybridization in the development of sugarcane varieties since the failure of early *S. officinarum* clones as a crop species has been to combine genes for cold, disease, and insect resistance and vigor from *S. spontaneum* with genes representing the best agricultural type from *S. officinarum*.

The fact that sugarcane is vegetatively propagated has permitted the use of heterozygous interspecific hybrid varieties for field production. This type of propagation system enables any desirable clone to be used and reproduced with exact precision regardless of its sexual reproductive capacity. This capacity in sugarcane has suffered a loss in efficiency because sugarcane is normally vegetatively reproduced under natural as well as cultivated conditions.

Another outstanding factor in the use of interspecific hybrids in sugarcane is the unusual feature of $2n+n$ chromosome behavior commonly observed when *S. officinarum* × *S. spontaneum* crosses are made. This curious phenomenon occurs in the original cross and in the first backcross, and results in the transmittal of the somatic rather than reduced chromosome complement of *S. officinarum* (used as the female parent in most cases) to the F_1 progeny. The reduced chromosome number from *S. spontaneum* is transferred normally. This unusual chromosome behavior has enabled the development of varieties with desirable agronomic

characteristics (from concentrated *S. officinarum* genes) coupled with increased disease resistance (from *S. spontaneum*) after only two to three backcrosses.

Today, breeding of new varieties of sugarcane is largely or wholly centered on intercrossing of preexisting hybrid commercial varieties. These varieties, usually used in biparental crosses, all have basically the same derivation. Their chromosome numbers range from 100 to 120, and there is no change from this range in the F_1 progeny. Furthermore, the fact that preferential chromosome pairing, or autosyndesis, is the rule for the chromosomes derived from different species that are contained in these commercial hybrids leads some researchers to believe that unless linkage groups are broken or new levels of ploidy are attained, no further outstanding improvements in varieties will be possible; in other words, a plateau may have been reached with the techniques in use today. The present practice is to periodically replace failing varieties that have succumbed to diseases and losses in productivity with new varieties derived in the same manner. This practice represents to some researchers an effort to maintain but not necessarily increase productivity. A solution to the static problem of variety improvement will perhaps be found in new work designed to broaden the genetic base upon which contemporary varieties are being bred for the U.S. mainland sugar-production areas.

Breeding Facilities

To breed sugarcane in the unfavorable climate of the Temperate Zone, it is necessary to circumvent such natural impediments to the work as wind, rain, and especially cold. All of these factors hinder or prevent the flowering of cane and thus the making of crosses. Facilities must be designed to provide optimum conditions for handling all stages of breeding work, from management of parental material in greenhouse flats through the last stage of processing juvenile seedlings under greenhouse conditions. Basically, the facilities should include a lightproof building for conducting photoperiod treatments, a tall greenhouse for carrying out various aspects of the breeding work, including crossing, outdoor racks for maintaining special can-cultured breeding stocks, and a standard greenhouse for seedling work. Specific details related to these facilities at Houma have been described in previous articles (Dunckelman 1973a, 1973b, 1974).

Photoperiod house

Ideally, this lightproof house should be large enough to contain several rooms for conducting more than one photoperiod treatment at any given time and tall enough to accommodate can-cultivated stools of breeding cane grown on rail carts; these canes may reach a height of 5 to 6 meters above ground level when tasseling. The rail carts may be equipped with electric- or gasoline-powered motors to move plants for exposure to direct sunlight when required if manual handling is too difficult. Normally, the house is not designed to fully expose cane to natural sunlight when plants are left inside.

The house may be constructed of any conventional building material. However, the large doors of such a facility should be contructed of lightweight material to prevent sagging and difficulty in opening and closing. The inside walls of the rooms, including door surfaces, should be insulated with lightweight material to help maintain optimum temperatures when the canes are inside. The house should be provided with a heating system equipped with thermostatic devices to control the inside temperature of the rooms during the cool nights of fall.

If one desires to supplement direct exposure to sunlight with artificial light, the rooms of the house must be equipped with batteries of lights suspended from the ceiling with pulleys, so that the lights can be raised or lowered to the desired distance from the flower-inductive spindle leaves of the growing plants. (The plants continue to grow in height until flowering starts.) The lights can be turned on and off automatically at the appropriate times by the use of timeclocks programed to change day lengths at a rate commensurate with natural or artificially contrived diurnal cycles that trigger the flowering process.

Breeding greenhouse

The greenhouse used to grow and protect breeding canes throughout the growing and

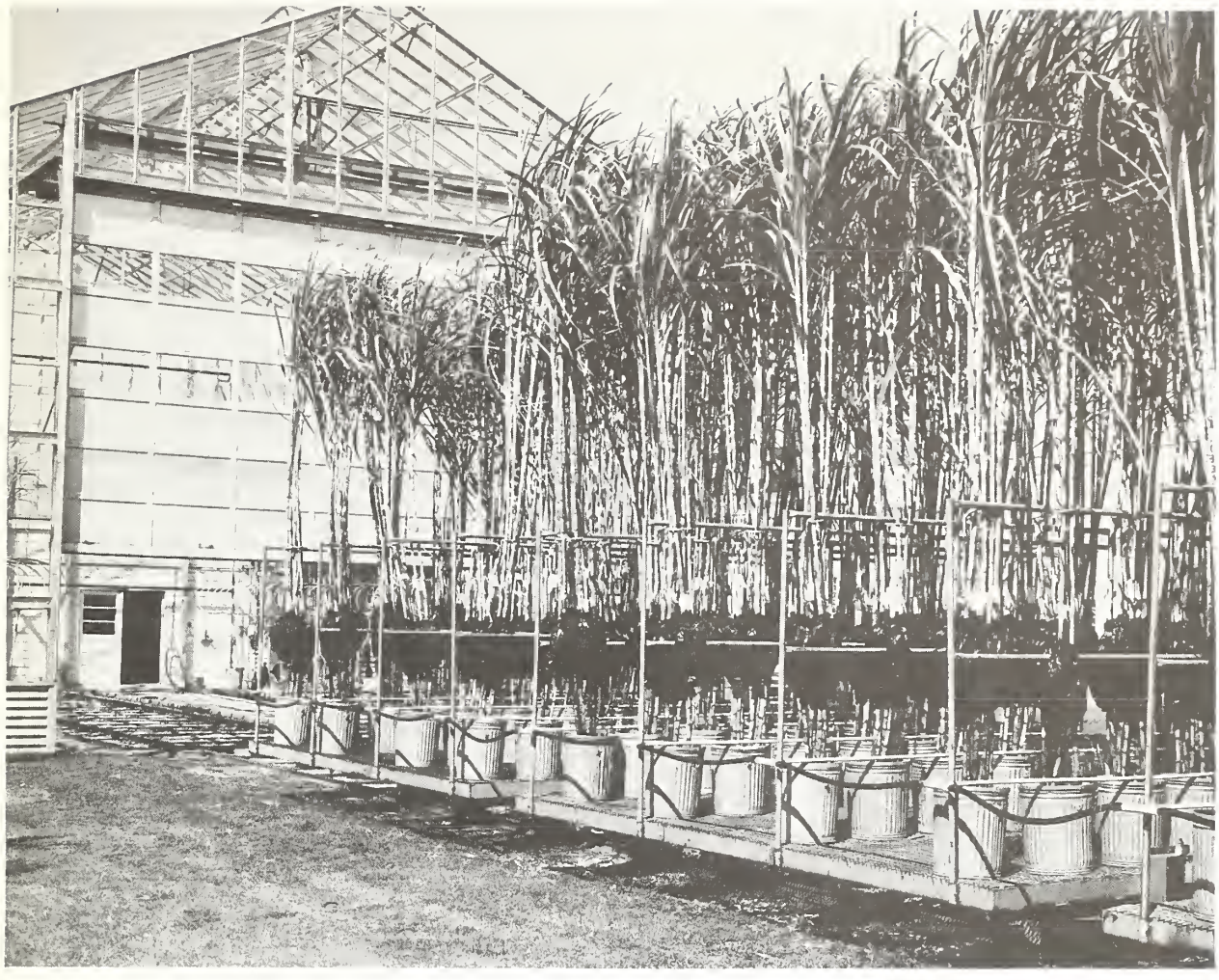

FIGURE 4.—Tall breeding greenhouse equipped with rail carts so that the cane can be moved easily in and out of the house. The black bulges just above the cans are airlayers; see figures 6 and 7 for a closer view.

flowering periods should, like the photoperiod house, be tall enough to accommodate the tallest flowering canes and large enough to accommodate the desired number of can cultures. It is wise to equip a facility of this type with rail carts (fig. 4), to move the breeding canes in and out of the house. Most canes will not flower if left under glass continuously; neither will they flower if left outdoors during the cool nights of fall when the natural photoperiod is conducive to flowering, but the cool nights are not.

For making crosses inside a greenhouse it is necessary to build isolation cubicles to minimize pollen contamination from one cross to the next (fig. 5). The framework for the cubicles can be constructed of wood or metal (preferably aluminum, for light weight), and the framework covered with plastic film. Each cubicle should be fitted with bars to which the canes can be tied at time of crossing.

If airlayered canes are used in crossing, it is essential that water troughs be provided to contain the rooted portion of the stalks when they are brought inside the greenhouse. The best troughs are permanently built of concrete, but removable troughs made of wood or metal may also be used. At Houma tapwater from the city system is used to fill the troughs, and no fertilizers or other chemicals are added to the water. A small electric- or gasoline-powered pump should be installed in the troughs to circulate the water; aerators can also be installed to freshen the circulating water.

Over portions of the troughs where there are

FIGURE 5.—Crosses in isolation cubicles of greenhouse.

no cubicles, tie bars of wood or metal, preferably the latter, should be installed. These may be used when canes are brought into the greenhouse in the boot or in the early tasseling stages since these canes must still be tied upright to maintain erectness; the bars can also be used to tie seed parents upright after removal from crosses.

A good heating system, preferably circulating hot water, must be installed in the greenhouse, along with thermostats to control the temperature at optimum levels, even during very cold periods. The opening and closing of the louvers at the top of the greenhouse, for best effect, should be thermostatically controlled. Side louvers at the lower part of the greenhouse can be regulated manually. The door(s) for the large opening through which the carts go in and out may be the overhead roll-up type or the sliding type mounted on rollers. Either type can be operated manually or electrically, as preferred.

Outdoor racks

Outdoor racks (fig. 6) may be constructed of wood or metal. Wood is cheaper and easier to use, but should be chemically treated to prevent rot. Bars to which to tie the canes upright may be nailed in permanent position or set in brackets so that they can be move higher up the posts as the canes grow taller. Posts should be set at least 1 meter in the ground to strongly stabilize the racks during hard winds, and should extend at least 1.5 to 1.8 meters above

FIGURE 6.—Outdoor racks. The worker is preparing an airlayer.

ground. The boards (laid on ground) on which to set the can cultures should be thick, wide boards treated with a wood preservative.

The outdoor racks are used to subject can cultures of early-flowering canes to a natural inductive photoperiod before the advent of cold nights of early fall. Can cultures of canes not included in field nurseries are also carried on the outdoor racks.

Seedling greenhouse

The seedling greenhouse, where the viability of seeds is tested, may be of more or less standard construction, with aluminum framework for durability and freedom from rust and rot. Benches should be made of heavy masonite material, and heat should be provided by hot-water pipes beneath. Automatic water sprinklers or misting systems, programed with timeclocks, may be installed above the benches to save time and labor in watering the thousands of seedlings grown each year.

AIRLAYERING

In the early fall, before flowering starts, about 1,600 mature stalks of sugarcane with apparent "ripeness to flower" are airlayered in cans (figs. 6 and 7). Airlayering is done to induce an aboveground system of roots on each stalk so that when the canes flower they can be cut below the rooted section and moved individually into the isolation cubicles of the tall greenhouse.

Several basal nodes of each stalk to be airlayered are first enclosed by tightly rolling a 61- by 61-cm piece of 10-mm polyethylene film around the stalk. The bottom of the film is secured tightly around the stalk with a wire bag tie tightened with a ratchet-type bag-tie tool. The top of the tubelike film is then rolled out by hand to make an opening of about 13 cm in diameter. Wet, unmilled sphagnum moss, which has been soaking in tubs of water, is then stuffed into the opening by hand and tamped tightly with a short section of broom handle (fig. 6). When the tube is filled to within 15 cm of the top, it is closed, and the top tightly secured with a wire bag tie. Airlayering should be done about 1 month in advance of tassel emergence. For the crossing program at Houma, approximately 1,600 airlayers are required. Two men can easily prepare 150 airlayers in an 8-hour day. If desired, a porous soil mixture can be used in place of the moss.

The airlayers are watered once a week with hoses equipped with pistol-grip nozzles fitted with a 30-cm length of 0.64-cm copper tubing sharpened at the end. The sharpened end of the

FIGURE 7.—Stalks of airlayered cane in can cultures. The worker is watering one of the airlayers.

copper pipe is easily thrust through the polyethylene film of the airlayers. With fingertip pressure on the trigger of the nozzle, water is injected into the airlayer in seconds (fig. 7). An insignificant puncture is left in the film of the airlayer after the pipe has been withdrawn, and each week the same puncture hole is used. With this method, two attendants can easily water 1,600 airlayers in 4 hours or less.

Three weeks after airlayering, extensive root systems have penetrated the moss, and thereafter, if necessary, the stalks of sugarcane can be cut from the cans and moved with minimum trauma. At Houma, all flowering stalks used in crosses, both male-sterile and male-fertile, are maintained on live roots. The plants will function normally throughout cross-pollination and seed maturation on roots induced by airlayering. This technique, plus greenhouse control measures, has resulted in the making of many desired crosses with excellent seed set per female tassel.

An alternative but less effective method than airlayering for maintaining individual flowering stalks during crossing is to place cut stalks in a weak acid solution. The technique, developed in Hawaii (Verret 1925, Manglesdorf 1953), does not, however, keep flowering stalks at peak vitality during the long period required to mature the seed following cross-fertilization.

CONTROL OF FLOWERING

Crossing for genetic improvement of sugarcane depends on flowering and production of true sexual seed. In Louisiana, climatic conditions for natural flowering range from partially satisfactory on Grand Isle to completely unsatisfactory at Houma and Baton Rouge. Nevertheless, progress in sugarcane-breeding technology during the last 30 years has permitted the production of practically unlimited quantities of true seeds under artificially controlled environmental conditions. The quantity of true seeds that can be produced is in nearly direct proportion to the number and size of photoperiod and greenhouse facilities. With the modest facilities at Houma, as many as 2,000 tassels and up to 500,000 true seeds are produced annually.

The inflorescence (arrow, tassel) of sugarcane is a large open panicle, the development of which has been described in minute detail by Artschwager et al. (1929) and Van Dillewijn (1952). In short, when a plant has reached a certain stage of development, its growing point may, under proper conditions, change from the vegetative to the reproductive stage. This means that the growing point ceases formation of leaf primordia and starts the formation of a flower primordium. Under the influence of suffi-

cient light, warmth, humidity, and favorable day length, the floral primordium will elongate, and eventually emerge from the boot stage as a nearly mature inflorescence composed of thousands of tiny flowers. And the flowers are perfect; that is, each has male organs (stamens) and female organs (pistils). Frequently, however, the flowers are sterile, the ovary being rudimentary and, more often, the anthers being without fertile pollen.

While many forms of *S. spontaneum* and its early-generation derivatives flower freely outdoors before the weather cools, practically all other sugarcane clones that normally flower later in the season rarely, if ever, produce tassels at this latitude. However, the essential conditions for the flowering of a large percentage of all kinds of sugarcane and related plants are artificially provided in the photoperiod house previously discussed. Flowers are produced early in the season, when the weather is still warm. The tall greenhouse provides controlled night temperatures later in the season when the weather begins to cool. It is during this time of year that the natural photoperiod becomes conducive to flowering, and many different kinds of sugarcane will flower and some will produce pollen if protected from low temperatures in greenhouses.

It has long been recognized that many breeding stocks required to broaden the genetic base of sugarcane do not flower at the same time even under the most favorable conditions. At Houma, variation in the time of flowering of both commercial and basic breeding stocks has been largely overcome by artificially contrived photoperiod and greenhouse treatments designed to either promote or retard the time of flowering and increase the efficiency of making the desired crosses. Two photoperiod regimens in particular have been found that produce tassels early in the season, while night temperatures are still high and day lengths too long for natural flowering in most breeding stocks. The most successful treatment is based on decreasing day lengths of specific duration, and the other, less widely used, involves a fixed day length that causes flowering in many breeding canes used at this location. The treatments may be started as early as midspring because at this time the can-cultured canes are nearly 5 months old and have reached a degree of maturity and size conducive to flowering. That is, provided that proper measures are taken to control day length, soil fertility, and soil moisture, temperature at this time of year is favorable both in the daytime and at night, and remains favorable for flowering until cool nights become a problem in the fall.

Both decreasing and fixed day lengths are used, not only to produce out-of-season flowers, but also to synchronize the flowering of clones that normally flower at widely different times. A decreasing day length (at the rate of 1 minute per day) starting at 12 hours 32 minutes and finishing at 11 hours 42 minutes has given excellent results in getting both commercial and basic breeding stocks to flower. The treatments can be staggered from midspring to midsummer to synchronize the flowering of selected parental material, thus enabling the breeder to make crosses that would otherwise be impossible. A fixed day length of 12 hours 25 minutes for 60 days or more has also been used with good results with various breeding canes. *S. officinarum* may require up to 80–100 recurring cycles of light and darkness to produce tassels.

Movement of can-cultured stools of cane from a cool to a warm environment, or vice versa, is also used to shorten or lengthen the flowering period of parent stocks. If stools are left outdoors during cool weather after flowering has started, the elongation and eventual emergence of tassels from the boot may be greatly delayed or prevented altogether. On the other hand, if cans of the same clone are moved into a warm greenhouse at intervals, flowering may be extended for several weeks. This technique is used primarily in the management of flowering and pollen production in many of the early-generation hybrids from new *S. spontaneum* lines; such hybrids usually set floral initials outside before the advent of cool nights at this station. Flowering canes resulting from late-summer photoperiod treatments are also moved in and out of the breeding greenhouse from mid to late fall to shorten or lengthen the time of flowering and to influence pollen production and viability; a cool environment enhances male sterility in some canes while a warm environment enhances male fertility. At optimum greenhouse temperatures for crossing (21°–32° C), some canes are normally male sterile while others are normally male fertile.

The emergence of the inflorescence marks the end of the development of the young inflores-

cence within the spindle or leaf sheath (boot) and the appearance of its visible floral buds. Most accounts of early breeding work considered flowering to be a single phenomenon, and the conditions which determined flowering were examined as to their effect on flowering as a whole. However, flowering is not a single phenomenon but a chain of physiological processes that can be separated into four major stages: floral initiation, floral organization, floral maturation, and emergence.

Opening of the flowers (anthesis) in relation to the emergence of the tassel from the boot influences the time when crosses are set up, and this aspect of flowering has been given much attention by breeders. In general, there is a substantial time lag between the first "pipping" of the tassel and the first opening of the flowers. However, wide variations have been noted at Houma. In some clones, anthesis begins almost as soon as the tassel starts to emerge from the boot, while in others the tassels may be fully expanded for a day or so to over a week before flowers begin to open. In a few clones, anthesis fails to occur in fully expanded, healthy-looking tassels even though they are kept in a warm greenhouse for up to 3 weeks after the tassel's emergence.

When flowers begin to open, it becomes necessary for the breeder to determine the "sex" of the parents to be crossed. This is done by observing the anthers, or pollen sacs, with a hand lens or microscope. If the anthers are yellow, shriveled, or papery, with unopened lobes, they contain little or no pollen and are invariably sterile. If the anthers are brownish or purple in color, plump, and exuding pollen grains from both lobes, they are usually fertile. However, in male-fertile clones the percentage of viable pollen and the amount of pollen produced varies among clones. Several tests can be used to determine pollen viability (see Moore 1975), but the best test for pollen fertility is germination in a suitable medium. However, the iodine test for starch in fresh pollen grains provides a quick method for estimating the percentage of viable pollen. Viable pollen grains are usually round and plump and will be stained a dark color by the iodine. Infertile grains are usually shriveled and will not become stained.

While it is possible to determine to a great extent the potential degree of male fertility, there is no practical or quick way to determine the degree of female (ovular) fertility in advance. The only practical way to test for ovular fertility is by repeated use in crosses with male-fertile clones. As a rule, under good conditions, healthy ovules of the flowering sugarcane plant are easily fertilized by the viable pollen from a different sugarcane plant. However, incompatibilities do sometimes exist between good pollen producers and good seed parents. Of interest also is that some strong pollen producers, while capable of fertilizing other clones, are incapable of self-fertilization, but this is the exception rather than the rule. Such self-incompatible clones can also be used as females in strictly biparental crosses and in polycrosses, but not in area crosses. In an area cross the influence of only one male on several different male-sterile clones is desired.

Male sterility may be due to one of two causes. The first is genetic, where through incompatibility of chromosome complements, pollen development is aborted. The second is largely environmental, where although some pollen may be produced, the anthers do not dehisce, and the pollen is not released. Varieties in the first category provide the most reliable female parents. In the second category, the sexuality of a variety may vary from time to time throughout the crossing season; a variety may produce viable pollen during one part of the season, while at other times, it may produce pollen that is largely or entirely infertile. A major factor affecting the sexuality of sugarcane is temperature. Male fertility is enhanced by temperatures above 22° C and suppressed by temperatures below 22° C.

Crossing and Production of True Seeds

We make all crosses in the isolation cubicles (fig. 5) in the tall sugarcane-breeding greenhouse. Crosses are biparental, usually of the area type, in which several different male-sterile clones are pollinated with a single male-fertile clone. As mentioned earlier, all flowering stalks to be crossed are live-rooted by airlayering in advance of the crossing period. Because of the detrimental effect of the high sodium content of the water at Houma on preservative solutions, none are used to keep flowering stalks alive, not even for males.

When flowering begins, the inflorescences are

carefully observed to determine the extent of anthesis and the condition of the florets, with special attention to the sexuality of the plants. These observations are made with the aid of a × 14 hand lens; this method has proved to be practical, quick, and quite satisfactory. If the anthers are plump, purplish or brownish in color, and exuding pollen from both lobes, the variety is used as a male; if the anthers are an insipid yellow, shriveled, small, and devoid of pollen, the variety is used as a female. However, in some cases, maleness or femaleness is not so clearly defined, and the breeder must decide whether or not a particular clone can be used as a male or female and under what crossing conditions.

In preparing to cross clones, anthesis in the males should have progressed far enough to insure a heavy production of pollen as soon as the cross has been set up. On the other hand, it is sound crossing procedure to use females in which anthesis is just beginning. If female tassels have been exposed to contamination from an undesired source, the part of the tassels with open flowers is removed. This is not to say that fully flowered tassels that have been exposed to outdoor temperatures of 10° C or lower for several days cannot be used in greenhouse crosses with strong males. When fertilized with heavy showers of viable pollen, they can produce large numbers of viable seeds if the ovaries and stigmatic surfaces are in good condition and highly receptive to the pollen.

In setting up crosses, it is important to arrange the flowering stalks in such a way that the pollen of the desired male will reach and effectively fertilize the ovules of the desired female. This is done in the warm, windless isolation cubicles of the crossing greenhouse by first affixing the male stalks to the tie bars and then arranging the female tassels in an advantageous position just beneath those of the male. Both male and female tassels must be spaced so that all flowers are exposed to falling pollen; clumping the tassels too tightly together can result in poor exposure of a majority of flowers, poor pollen distribution, and consequently poor seed set. Throughout the crossing season greenhouse temperatures are kept within 21° to 32° C. Sufficient humidity is retained in the greenhouse during bright, sunny weather by frequently hosing down the floor.

Where the flowering parent clones are carefully arranged within the crossing cubicles, there is some natural shedding of pollen during anthesis and some cross-fertilization undoubtedly takes place, but there is probably very little pollen that is shed naturally in the windless environment of the crossing house. It is therefore essential that the metal tie bars in the cubicles be briskly tapped each morning to insure maximum pollen dispersal and transference from the males to the females. Tapping should be done daily during anther dehiscense, when the flowers of both male and female tassels are open. The best time to tap crosses in the greenhouse is between 7:30 and 10:30 in the morning, depending to some extent on humidity. If humidity is high (during cloudy, rainy spells), pollen release may be delayed and reduced to some extent, but if the weather is dry and sunny, the humidity inside the greenhouse is lower, and anther dehiscense and pollen dispersal is enhanced during the early part of the day.

The duration of pollination is variable, and depends primarily on the duration of anthesis in the parent clones. Some flowers open each day during early morning hours, and 10 days or more may be required for completion of the process. At Houma, females and males are sometimes left in crosses for as long as 2 weeks. Repeated showers of pollen on female tassels for a few days after all the flowers are open increases the chance of more complete fertilization of the thousands of flowers in a female tassel, and this technique has undoubtedly contributed to the excellent seed set commonly experienced at this location. Ordinarily, after 2 weeks in the cubicles, the crosses are dismantled and the males are discarded. The female stalks are carefully removed from the cubicles and bound together with wire bag ties with a ratchet-type bag-tie tool, and the tassels are bagged with large paper laundry bags (fig. 8). The tops of the bags are finely perforated to prevent overheating. The bagged seed parents are then tied upright to metal tie bars inside the greenhouse on the side opposite the crossing cubicles.

Seed development and maturation in sugarcane are progressive processes and follow the pattern of anthesis. The first flowers open at the ends of individual bracts, or rachises, at the top of the tassel, and these are the first to be fertilized if viable pollen is introduced. Opening of the flowers continues inward and downward for

FIGURE 8.—Bagged tassels of seed parents.

up to 2 weeks in the average tassel, until the bottommost flowers finally open and become receptive to pollination. When the seeds at the top of the tassel become ripe and fall, those at the bottom of the tassel are still developing. If the tassel is harvested when the top seeds first begin to disperse, progressively immature seeds in all other stages of development will be weakened or lost. If the seed parents are bagged, the ripe seeds will fall in the bags instead of to the greenhouse floor. The ripening process is usually complete 16 to 20 days after bagging. The bags may be lowered and opened from time to time to observe the progress of seed maturation.

When seed maturation is as complete as possible, the peduncles of the seed parent are cut just below the mouth of the paper bag. The crossing tag, with cross number, parentage, number of female tassels, and date of cross, is detached from the stalks and tied to the string at the mouth of the bag. The bag is then hung in the wooden drying cabinet in the headhouse. About 20 crosses can be dried at a time. Heat from an electric heater rises through large perforations in the cabinet floor and exits through the top of the cabinet, which is also perforated. The dryer is kept at about 38° C for 72 hours. At this time the bags are removed from the cabinet and the seed-bearing "fuzz" is hand-stripped from the tassels. The fuzz is mixed thoroughly to distribute viable seeds as uniformly as possible. A 1-gram sample is taken in random pinches so as to get a representative sample of seeds for a germination test, and the fuzz is then dumped from the large individual laundry bags into a metal washtub and transferred to small paper bags identified with the cross number and parentage. The original crossing tag is placed inside the small bag with the fuzz. The bags are weighed, and the weight of fuzz is recorded on the bags and in the crossing record.

The 1-gram test sample of fuzz is planted in small blocks marked out with separators in metal flats placed on a bench in one of the seedling greenhouses. The soil in the flats is sterilized with steam to kill any organisms and weed seeds that may be present. Each sample of fuzz is carefully numbered and evenly spread in the small blocks. The thinly spread fuzz is then watered gently with a sprinkler and covered lightly with finely screened soil. The surface of the soil is again sprinkled lightly with water, and fiberglass covers are placed on the flats. The covers are removed during the day to avoid excessively high temperature that might scald and kill germinating seeds. At night, for the first 3 days, the flats are covered. At the end of 2 or 3 days, the green coleoptiles of the tiny sugarcane plants begin to come through the soil, and the flats are left uncovered. After the fifth day, a preliminary germination count is made, and the number of viable seeds per cross is estimated.

The estimate is made by multiplying the estimated number of seedlings per 1-gram sample by the number of grams of fuzz in the entire cross. The crosses are then given to the agronomist in charge of the seedling phase of the breeding program. Six weeks after planting the germination tests, the seedlings from the 1-gram samples of fuzz are pulled from the flats and counted to get a better estimate of total number of seedlings per cross.

Storage of True Seeds

An experiment showed that seeds stored at -17.8° C kept better than seeds stored at room temperature or at 7.2° to 10° C in an electric refrigerator (Abbott 1950). After this discovery, it has been routine practice to store excess sugarcane seeds (once dried) at below freezing temperatures, and it is now known that these seeds will remain viable for several years. This is very advantageous since each year more true seeds are produced from crosses at Houma than can be grown in one season.

Usually, large portions of seeds from the most highly desired crosses are germinated each season, while smaller portions of seeds are germinated from crosses of completely unknown but promising parentage. All seeds not germinated are then stored in the freezer for future use. After determining the value of seedling progenies in the field, the breeders can go back to the freezer for more seeds of the most outstanding crosses to be planted the next year. Conservation of true seeds may not be of great importance when several good breeding seasons occur in succession, but in years when little or no seeds are produced, seeds saved from previous seasons may be used to fill in the deficiency.

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